Report for 2003OR31B: Environmental Analysis of Wastewater Effluents and Biosolids-derived Endocrine Disuptiong Chemicals in the Willamette River

- Conference Proceedings:
  - Kassim TA, 2004, Forensic analysis, mass balance and fate modeling of endocrine disrupting chemicals in wastewater treatment plants. 4th SETAC World Congress, Society of Environmental Toxicology and Chemistry, SESSION: A05 Endocrine-Disrupting Chemicals in Wastewater Treatment Effluents, 14-18 November, 2004, Portland, Oregon
  - Kassim, T.A., 2004, Multicomponent joint toxic effect modeling of organic contaminates from complex mixtures. 4th SETAC World Congress, Society of Environmental Toxicology and Chemistry, SESSION: B34 Toxicity of Complex Mixtures, 14-18 November, 2004, Portland, Oregon
  - Kassim, T.A., 2004, Forensic Analysis of Wastewater Effluents- and Biosolids-Derived Endocrine Disrupting Chemicals in the Willamette River. Gordon Research Conference, Environmental Sciences: Water, June 27 - July 2, 2004, Holderness School, Plymouth, NH
  - Kassim, T.A., 2002, Environmental Analysis and impact assessment of endocrine disrupters in the Willamette River Project approach. The 5th International Conference of the Balkan Environmental Association on Transboundary Pollution, 7-10 Nov. 2002, Belgrade, Yugoslavia
  - Kassim, T.A., 2004, Sorption/Dissipation Behavior of Veterinary and Human Antibiotics in Solid-Phase Environments. Gordon Research Conference, Environmental Sciences: Water, June 27 - July 2, 2004, Holderness School, Plymouth, NH

Report Follows

Background Information:

An environmental endocrine disrupter is defined as an external compound that interferes with or mimics natural hormones in the body that are responsible for the maintenance, reproduction, development, and/or behavior of an organism (Folmar, 1993; Fry and Toone, 1981; Goodbred et al., 1996; Jobling et a., 1996; Schmitt et al., 1990; Smith et al., 1997; U.S. Environmental Protection Agency, 1997). Hypotheses about which chemicals may be endocrine disrupters, about the mechanisms through which they operate, and about which animals may be affected have been discussed in numerous publications (Kassim and Simoneit, 2001; Bevans et al., 1996; Colborn and Clement, 1992; Henny et al., 1996; Facemire et al., 1995; Guillette et al., 1994; Davis and Bortone, 1992; EPA, 1997); however, few regional or national studies related to assessment and water quality have been conducted to test these hypotheses.

The U.S. Geological Survey (USGS) National Water Quality Assessment (NAWQA) program recently found evidence of endocrine disruption in common carp (*Cyprinus carpio*) and largemouth bass (*Micropterus salmoides*) collected from waterways that contain synthetic organic compounds. Various synthetic chemicals from several chemical groups have been identified as potentially having endocrine disrupting effects (Kassim and Simoneit, 2001). Some of these chemicals have the potential to cause reproductive impairment in aquatic organisms (Colborn and Clement, 1992; Henny et al., 1996; Facemire et al., 1995). Alteration in blood concentrations of sex steroid hormones and vitellogenin may be associated with reproductive impairment and other critical reproductive factors (Guillette et al., 1994; Davis and Bortone, 1992; EPA, 1997). Evidence indicates concentrations of sex steroid hormones (estrogen and testosterone) and vitellogenin (egg protein produced by females) were different in fish from contaminated and reference sites.

The generation and disposal of biosolids (used as soil amendments) and wastewater effluents produced at municipal wastewater treatment plants (WWTPs) are considered to be the main sources of EDCs' in the aquatic environment (Horwitz et al., 1996; Kendall et al., 1998; Ssnyder et al., 2000; USEPA, 1997). For example, approximately 900 kg of biosolids on a dry basis are produced from the treatment of 1 million gallons of wastewater (Ahlborg et al., 1995; Carlsen et al., 1995). These solids are typically dewatered on site and disposed of at landfills, incinerators or on agricultural fields. Disposal of sewage sludge on agricultural fields recycles the nutrients captured from municipal wastewater into agricultural soils. However, biosolids applied as soil amendments can contain significant quantities of EDCs derived from the municipal wastewater or organic metabolites produced during WWTPs. These organics have the potential to adversely impact soil receiving the biosolids, surface and groundwater in the vicinity of application, on crops grown on sludge-amended soils, and on animals and humans that may consume the crops grown on the soils.

Local State Problem Area:

The Willamette River (Figure 1) is the 10th largest river in the United States and the heart of Oregon. In the 1930s, the Willamette was so polluted that fish were dying and the water was no longer safe for human use. Decades of local effort resulted in significant improvements to water quality and, by the 1970s, the Willamette became a model for what concerned citizens can accomplish in environmental restoration. Recently, another critical milestone in the history of the Willamette River has approached. A study performed in 1997 by the US-EPA and the Oregon-DEQ found that the aquatic environment of the river is contaminated with organic pollutants that causes several fish species to die or have deformation.

A recent investigation by the USGS National Water Quality Assessment (NAWQA) program has found that several fish species are dying or have deformations, as well as evidence of endocrine disruption in common carp and largemouth bass collected from the river. In additon, another study performed in 2001, carried out by the Principal Investigator and funded by the USGS, has indicated the presence of several EDCs in the river (Kassim, 2002).

Research Objectives and Approach:

The current effort to investigate the occurrence and characterization of a comprehensive list of EDCs in both effluents and biosolids disposed from and/or generated in three major wastewater treatment plants (WWTPs) will help complement the understanding of EDCs' chemodynamics and their control in the Willamette River. This investigation was accomplished through the successful completion of the following tasks:

TASK 1 "COMPILATION OF RELEVANT RESEARCH": A literature search was conducted to identify the most common OWCs present in US streams and rivers and their concentration ranges. The search did also aid in compiling relevant data regarding chemical, physical and toxicological characteristics for these OWCs.

TASK 2 "AN ENVIRONMENTAL FORENSIC/MOLECULAR MARKER APPROACH": This provided the first comprehensive statewide investigation of the occurrence, characterization and source confirmation of a broad suite of organic wastewater contaminants (OWCs) of emerging environmental concern in two major sources (e.g., effluents and biosolids) in the Willamette River. These OWCs included antibiotics, other prescription and nonprescription drugs, steroids, reproductive hormones, personal care products, products of oil use and combustion, and other extensively used chemicals. The target OWCs were selected (see Table 1) because they are:

- Expected to enter the Willamette River environment through common wastewater effluent or biosolids (as soil amendments) pathways,
- Used in significant quantities in Oregon,
- Have human or environmental health implications,
- Are potential indicators of certain classes of compounds or sources, and/or
- Can be accurately measured using available technologies.

Although OWCs are just a small subset of the compounds being used by society, they represent a starting point for this synergistic investigation that examines their occurrence in the Willamette River.

TASK 3 "STATISTICAL ANALYSIS OF DATA": Data collected were statistically examined in order to determine any significant interaction of the variables. The test results were analyzed using quantitative methods to summarize the information hidden in the multivariate data sets. This reduced the complex data matrix into a simpler form without distorting its information content and thus, the conclusions became more readily inferable. Analysis tools included multivariate analysis of variance, correlation analysis between variables, multiple regression analysis, linear programming and principal component analysis.

TASK 4 "FINAL RESEARCH REPORT": Summary of test results and analyses performed were provided electronically for review and feedback.

Methods, Procedures, and Facilities:

The present project used the state of knowledge and information in the fields of environmental organic chemistry, environmental engineering, and computer science/programming. This required the use of state of the art instruments and latest modeling techniques.

Sampling: Both biosolids and wastewater effluents were collected from three major wastewater treatment plants (WWTPs) located on the Willamette River (Figure 1) . These WWTPs locations include Corvallis, Albany, and Salem.

Characterization and Identification of OWCs: The following sections summarize the approach used to characterize and identify the molecular compositions of OWCs in both effluents samples and biosolids, as follows:

Extraction and separation

An extraction protocol originally developed and revised by Kassim (1994; 1998) and Kassim and Simoneit (1995b) were further modified and verified for the qualitative and quantitative analyses of different organic classes found in both effluent and biosolids samples (Kassim and Simoneit, 2001). In brief, effluent samples were liquid/liquid extracted with methylene chloride-methanol (2:1). Biosolids were extracted in a Soxhlet apparatus with methylene chloride-methanol (2:1). All the extracts (EOM) were concentrated to 2 ml and hydrolyzed overnight with 35 ml of 6% KOH/methanol. The corresponding neutral and acidic fractions were successively recovered with *n*-hexane (4x30 ml), the latter after acidification (pH 2) with 6*N* HCl. The acidic fractions, previously reduced to 0.5 ml, will be esterified overnight with 15 ml of 10% BF$_3$/methanol. The BF$_3$/methanol complex were destroyed with 15 ml of water, and the methyl esters were recovered by extraction with 4x30 ml of *n*-hexane. The neutrals were fractionated by long column chromatography. The following fractions were collected:

    (I)   45 ml of *n*-hexane (aliphatic hydrocarbons, F1),
    (II)   25 ml of 10% methylene chloride in *n*-hexane (monoaromatic hydrocarbons "MAHs", F2),

(III)	40 ml of 20% methylene chloride in *n*-hexane (polycyclic aromatic hydrocarbons "PAHs", F3),
(IV)	25 ml of 50% methylene chloride in *n*-hexane (esters and ketones, F4),
(V)	25 ml of methylene chloride (ketones and aldehydes, F5), and
(VI)	50 ml of 10% methanol in methylene chloride (alcohols, F6).

The last fraction and an aliquot of the total extract were derivitized prior to gas chromatographic-mass spectrometric (GC-MS) analysis for further qualitative molecular examination by silylation with bis(trimethylsilyl)trifluoroacetamide. A recovery experiment for the long column chromatography were carried out using several deuterated standards.

Instrumental analyses

All samples were analyzed by gas chromatography (GC), GC-ECD, GC-MS. The GC-MS analyses of the samples were performed using a Hewlett-Packard 5973 MSD quadrupole mass spectrometer operated in the electron impact mode at 70eV and coupled to an HP Model 6890 gas chromatograph. The GC was equipped with a 30 m x 0.25 mm i.d capillary column coated with DB-5 (J & W Scientific, film thickness 0.25 μm) and operated using helium as carrier gas.

Identification and quantification

Compound identification was based on comparison with the GC retention times and/or mass fragmentation patterns of standard reference materials and with the help of the Library Molecular marker identification tabulated as follows: (a) *Positive,* when the sample mass spectrum, authentic standard compound mass spectrum, and their retention times agrees well; (b) *Probable,* same as above except no standards are available, but the sample mass spectrum agrees very well with the standard library; (c) *Possible,* same as above except that the sample spectrum contains information from other compounds but with minor overlap; and (d) *Tentative,* when spectrum contains additional information from possibly several compounds with overlap. Identification and response factors of several EDCs were determined using a suite of standard compounds. Quantification was based on the application of per-deuterated compounds for each respective EDC fraction as internal standards.

Organic carbon analysis:

Organic carbon analyses were carried out for all effluent and biosolids samples using a Carlo Erba NA-1500 CNS analyzer. The concentrations of all EDCs were calculated relative to the total organic carbon (TOC) content of the samples.

Characterization and Genetic-Source Partitioning

EDCs data and other chemical indices of the compositions of both effluents and biosolids were examined statistically in order to determine any significant environmental variations, and to construct a source-partitioning model specific for the Willamette. All statistical analyses and modeling approaches were performed using extended Q-mode factor analysis, linear programming and artificial intelligence-neural network programs and verified using Monte Carlo Simulation.

Facilities: The analyses were conducted in the Environmental Engineering Laboratory at Oregon State University. Adequate chemical laboratory space and the following instrument facilities for organic chemical identification and characterization are present under the direction of or accessible to the principal investigator. This includes: (a) a temperature programmable gas chromatograph coupled to a Chemstation data system (Hewlett-Packard 5890) for high molecular weight compound analyses, (b) one Hewlett-Packard 5973 MSD quadrupole GC-mass spectrometer coupled to a Chemstation data system, (c) elemental (CHN) analysis, (d) temperature controlled environmental chambers, (e) sample collection and coring equipment, and (f) multimedia modeling software.

Principal Findings and Significance:

The current pollution in the Willamette River is the result of anthropogenic activities. The present investigation indicated the presence of several chemicals of complex organic mixtures (COMs) or possible organic wastewater contaminants (OWCs) (see Tables 1 and 2).

The preliminary investigation indicated the occurrence of various toxic and endocrine disruptive complex organic mixtures (COMs) or organic wastewater contaminants (OWCs) in the Willamette River's surface water, and various WWTP-effluents and –biosolids for Willamette River treatment facilities. In general, WWTP-treated effluents and surface water samples from the Willamette River showed high concentrations of a large number of COMs/OWCs. This includes the following: 78 ug/L of total aliphatic hydrocarbons (e.g., *n*-alkanes, isoprenoids, UCMs, terpanes, diasteranes, and steranes); 230 ng/L of total polycyclic aromatic hydrocarbons (e.g., neutral and alkyl-substituted

compounds); 92 ng/L of veterinary and human antibiotics; 47 ng/L of non-prescription drugs (e.g., codeine and caffeine); 63 ng/L total phthalate esters; 74 ng/L bisphenol A; 180 ng /L nonylphenol; 125 ng/L sex steroid hormones and their metabolites (e.g., 17β-estradiol, 17α-ethinylestradiol, mestranol, estrone, estriol, cholesterol, coprostanol and epi-coprostanol).
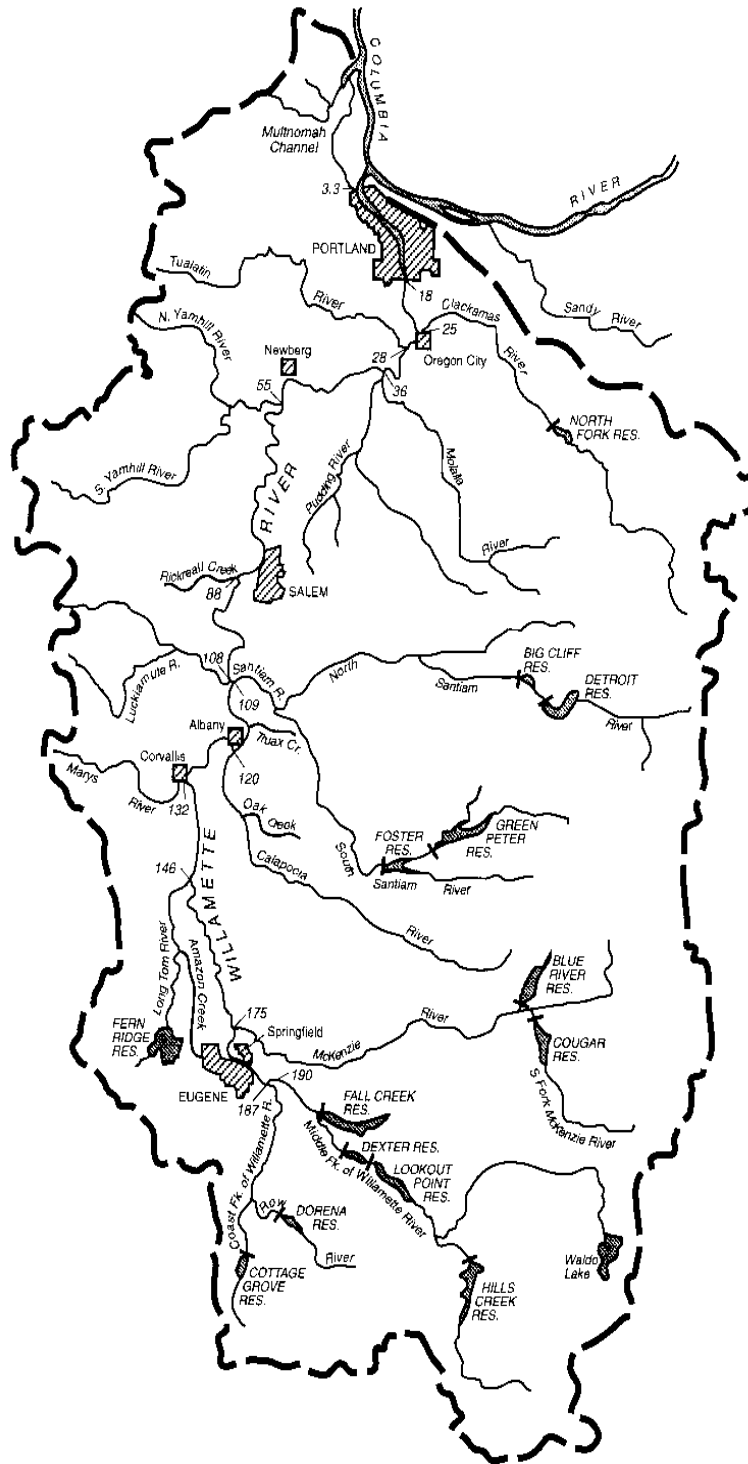
Figure 1. The Willamette River, Oregon

Table 1. Contaminant Name, Chemical Composition and Molecular Weight representing the Willamette River Environment.

#	COMPOUND NAME	CHEMICAL COMPOSITION	MW	#	COMPOUND NAME	CHEMICAL COMPOSITION	MW
	**(I) ALIPHATIC HYDROCARBONS**				**(II) POLYCYCLIC AROMATIC HYDROCARBONS**		
	n-Alkanes				**Neutral PAHs**		
1	n-hexadecane	$C_{16}H_{34}$	226	69	Quinoline "benzo[b]pyridine"	C_9H_7N	129
2	n-heptadecane	$C_{17}H_{36}$	240	70	2,3-Dimethyl quinoline	$C_{11}H_{11}N$	157
3	n-octadecane	$C_{18}H_{38}$	254	71	Phenanthrene	$C_{14}H_{10}$	178
4	n-nonadecane	$C_{19}H_{40}$	268	72	Anthracene	$C_{14}H_{10}$	178
5	n-eicosane	$C_{20}H_{42}$	282	73	Fluoranthene	$C_{16}H_{10}$	202
6	n-heneicosane	$C_{21}H_{44}$	296	74	Pyrene	$C_{16}H_{10}$	202
7	n-docosane	$C_{22}H_{46}$	310	75	11 H-Benzo[a]fluorene	$C_{17}H_{12}$	216
8	n-tricosane	$C_{23}H_{48}$	324	76	Benz[a]anthracene	$C_{18}H_{12}$	228
9	n-tetracosane	$C_{24}H_{50}$	338	77	Chrysene/triphenylene	$C_{18}H_{12}$	228
10	n-pentacosane	$C_{25}H_{52}$	352	78	Benzo[b+k]fluoranthenes	$C_{20}H_{12}$	252
11	n-hexacosane	$C_{26}H_{54}$	366	79	Benzo[e]pyrene	$C_{20}H_{12}$	252
12	n-heptacosane	$C_{27}H_{56}$	380	80	Benzo[a]pyrene	$C_{20}H_{12}$	252
13	n-octacosane	$C_{28}H_{58}$	394	81	Perylene	$C_{20}H_{12}$	252
14	n-nonacosane	$C_{29}H_{60}$	408	82	Indeno[1,2,3-cd]pyrene	$C_{22}H_{12}$	276
15	n-triacontane	$C_{30}H_{62}$	422	83	Dibenz[ah]anthracene	$C_{22}H_{14}$	278
16	n-hentriacontane	$C_{31}H_{64}$	436	84	Benzo[ghi]perylene	$C_{22}H_{12}$	276
17	n-dotriacontane	$C_{32}H_{66}$	450	85	Anthanthrene	$C_{22}H_{12}$	276
18	n-tritriacontane	$C_{33}H_{68}$	464	86	Coronene	$C_{24}H_{12}$	300
19	n-tetratriacontane	$C_{34}H_{70}$	478	87	Dibenzo[ae]pyrene	$C_{24}H_{14}$	302
20	n-pentatriacontane	$C_{35}H_{72}$	492		**Alkyl-Substituted PAHs**		
21	n-hexatriacontane	$C_{36}H_{74}$	506		*(Alkyl phenanthrene series)*		
22	n-heptatriacontane	$C_{37}H_{76}$	520	88	3-Methylphenanthrene (3MP)	$C_{15}H_{12}$	192
23	n-octatriacontane	$C_{38}H_{78}$	534	90	2-Methylphenanthrene (2MP)	$C_{15}H_{12}$	192
24	2,6,10-trimethylpentadecane (norpristane)	$C_{18}H_{38}$	254	91	9-Methylphenanthrene (9MP)	$C_{15}H_{12}$	192
25	2,6,10,14-tetramethylpentadecane (pristane)	$C_{19}H_{40}$	268	92	1-Methylphenanthrene (1MP)	$C_{15}H_{12}$	192
26	2,6,10,14-tetramethylhexadecane (phytane)	$C_{20}H_{42}$	282	93	Dimethylphenanthrenes	$C_{16}H_{14}$	206
27	Unresolved Complex Mixture (UCM)			94	Trimethylphenanthrenes	$C_{17}H_{16}$	220
	Tricyclic terpanes			95	Tetramethylphenanthrenes	$C_{18}H_{18}$	234

#	NAME	CHEMICAL COMPOSITION	MW
28	C$_{19}$-tricyclic	C$_{19}$H$_{34}$	262
29	C$_{20}$-tricyclic	C$_{20}$H$_{36}$	276
30	C$_{21}$-tricyclic	C$_{21}$H$_{38}$	290
31	C$_{23}$-tricyclic	C$_{23}$H$_{42}$	318
32	C$_{24}$-tricyclic	C$_{24}$H$_{44}$	332
33	C$_{25}$-tricyclic	C$_{25}$H$_{46}$	346
34	C$_{26}$-tricyclic (S)	C$_{26}$H$_{48}$	360
35	C$_{26}$-tricyclic (R)	C$_{26}$H$_{48}$	360
36	C$_{28}$-tricyclic	C$_{28}$H$_{50}$	388
37	C$_{29}$-tricyclic	C$_{29}$H$_{52}$	402
Tetracyclic terpanes			
38	C$_{24}$-tetracyclic (17,21-seco-hopane)	C$_{24}$H$_{42}$	330
39	C$_{28}$-tetracyclic (18,14-seco-hopane)	C$_{28}$H$_{50}$	386
40	C$_{29}$-tetracyclic (18,14-seco-hopane)	C$_{29}$H$_{52}$	400
Pentacyclic triterpanes			
41	18α(H)-22,29,30-trisnorneohopane (Ts)	C$_{27}$H$_{46}$	370
42	17α(H)-22,29,30-trisnorhopane (Tm)	C$_{27}$H$_{46}$	370
43	17α(H),21β(H)-29-norhopane	C$_{29}$H$_{50}$	398
44	17α(H),21β(H)-hopane	C$_{30}$H$_{52}$	412
45	17α(H),21β(H)-homohopane (22S)	C$_{31}$H$_{54}$	426
46	17α(H),21β(H)-homohopane (22R)	C$_{31}$H$_{54}$	426
47	17α(H),21β(H)-bishomohopane (22S)	C$_{32}$H$_{56}$	440
48	17α(H),21β(H)-bishomohopane (22R)	C$_{32}$H$_{56}$	440
49	17α(H),21β(H)-trishomohopane (22S)	C$_{33}$H$_{58}$	454
50	17α(H),21β(H)-trishomohopane (22R)	C$_{33}$H$_{58}$	454
51	17α(H),21β(H)-tetrakishomohopane (22S)	C$_{34}$H$_{60}$	468
52	17α(H),21β(H)-tetrakishomohopane (22R)	C$_{34}$H$_{60}$	468
53	17α(H),21β(H)-pentakishomohopane (22S)	C$_{35}$H$_{62}$	482
54	17α(H),21β(H)-pentakishomohopane (22R)	C$_{35}$H$_{62}$	482
Diasteranes			
55	13α,17β-diacholestane (20S)	C$_{27}$H$_{48}$	372

#	NAME	CHEMICAL COMPOSITION	MW
	(Alkyl pyrene/fluoranthene series)		
96	Methylpyrenes/fluoranthenes	C$_{17}$H$_{12}$	216
97	Dimethylpyrenes/fluoranthenes	C$_{18}$H$_{14}$	230
98	Trimethylpyrenes/fluoranthenes	C$_{20}$H$_{16}$	244
	(Alkyl 228 series)		
99	Methyl-228	C$_{19}$H$_{14}$	242
100	Dimethyl-228	C$_{20}$H$_{16}$	256
	(Alkyl 252 series)		
101	Methyl-252	C$_{21}$H$_{14}$	266
102	Dimethyl-252	C$_{22}$H$_{16}$	280
103	Trimethyl-252	C$_{23}$H$_{18}$	294
104	Tetramethyl-252	C$_{24}$H$_{20}$	308
(III) NON-HYDROCARBONS			
Phthalates			
105	Phthalic anhydride	C$_8$H$_4$O$_3$	148
106	Dimethyl phthalate	C$_{10}$H$_{10}$O$_4$	194
107	Diethyl phthalate	C$_{12}$H$_{14}$O$_4$	222
108	Bis(2-ethylhexyl) phthalate	C$_{24}$H$_{38}$O$_4$	390
Phenols and Substituted Phenols			
109	Phenol	C$_6$H$_6$O$_2$	94
110	2,3,4,5,6-Pentachlorophenol	C$_6$Cl$_5$OH	266
Amines			
111	N,4-Dimethylbenzenamine	C$_8$H$_{11}$N	121
112	N,N, 3-Trimethylbezenamine	C$_9$H$_{13}$N	134
Amides			
113	N-(2, 4-Dmethylphenyl)formamide	C$_9$H$_{11}$NO	149
Various Alcohols			
114	Dicyclopentadieneol	C$_{10}$H$_{13}$O	149
115	[1,1' - Biphenyl]-2-ol	C$_{12}$H$_{10}$O	170
Acids			
116	Benzoic acid	C$_7$H$_6$O$_2$	122

#	COMPOUND NAME	CHEMICAL COMPOSITION	MW	#	COMPOUND NAME	CHEMICAL COMPOSITION	MW
56	13α,17β-diacholestane (20R)	$C_{27}H_{48}$	372	117	Nonanoic acid	$C_9H_{18}O_2$	158
	Steranes			118	Decanoic acid	$C_{10}H_{20}O_2$	172
57	5α,14α,17α-cholestane (20S)	$C_{27}H_{48}$	372	119	Dodecanoic Acid	$C_{12}H_{24}O_2$	200
58	5α,14β,17β-cholestane (20R)	$C_{27}H_{48}$	372	120	Tetradecanoic acid	$C_{14}H_{28}O_2$	228
59	5α,14β,17β-cholestane (20S)	$C_{27}H_{48}$	372	121	Hexadecanoic acid	$C_{16}H_{32}O_2$	256
60	5α,14α,17α-cholestane (20R)	$C_{27}H_{48}$	372				
61	5α,14α,17α-ergostane (20S)	$C_{28}H_{50}$	386				
62	5α,14β,17β-ergostane (20R)	$C_{28}H_{50}$	386				
63	5α,14β,17β-ergostane (20S)	$C_{28}H_{50}$	386				
64	5α,14α,17α-ergostane (20R)	$C_{28}H_{50}$	386				
65	5α,14α,17α-sitostane (20S)	$C_{29}H_{52}$	400				
66	5α,14β,17β-sitostane (20R)	$C_{29}H_{52}$	400				
67	5α,14β,17β-sitostane (20S)	$C_{29}H_{52}$	400				
68	5α,14α,17α-sitostane (20R)	$C_{29}H_{52}$	400				

Table 1: List of Organic Wastewater Contaminants (OWCs) detected and characterized in the present project.

	Organic Wastewater Contaminants of Complex Mixtures		
Veterinary and Human Antibiotics	**Tetracyclines** Chlortetracycline Doxycycline Oxytetracycline Tetracycline **Fluoroquinolones** Ciprofloxacin Enrofloxacin Norfloxacin Sarafloxacin	**Macrolides** Erythromycin-H_2O (*metabolite*) Tylosin Roxithromycin **Sulfonamides** Sulfachlorpyridazine Sulfamerazine Sulfamethazine Sulfathiazole	Sulfadimethoxine Sulfamethiazole Sulfamethoxazole **Others** Lincomycin Trimethoprim Carbadox Virginiamycin

Human Drugs	**Prescription**
	Antacid
	   Cimetidine
	    Ranitidine
	antidepressant
	   Fluoxetine
	   Paroxetine
	Antihypertensive
	   Enalaprilat
	   Diltiazem
		Metformin (*antidiabetic agent*)
		
		Digoxin
		Warfarin (*anticoagulant*)
		Salbutamol (*antiasthmatic*)
		Gemfibrozil (*antihyperlipidemic*)
		Dehydronifedipine (*antianginal metabolite*)
		Digoxigenin (*digoxin metabolite*)
			Non-Prescription
			Acetaminophen (*analgesic*)
			Ibuprofen (*anti-inflammatory, analgesic*)
			Codeine (*analgesic*)
			Caffeine (*stimulant*)
			1,7-Dimethylxanthine (*caffeine metabolite*)
			Cotinine (*nicotine metabolite*)

Industrial and Household Wastewater Products	**Insecticides**
	Diazinon
	Carbaryl
	Chlorpyrifos
	cis-Chlordane
	N,N-diethyltoluamide
	Lindane
	Methyl parathion
	Dieldrin
	
	Plasticizers
	bis(2-Ethylhexyl)adipate
	Ethanol-2-butoxy-phosphate
	bis(2-Ethylhexyl)phthalate
	Diethylphthalate
	Triphenyl phosphate
	
	Detergent metabolites
	p-Nonylphenol
		Nonylphenol monoethoxylate
		Nonylphenol diethoxylate
		Octylphenol monoethoxylate
		Octylphenol diethoxylate
		
		Fire retardants
		
		Tri(2-chloroethyl)phosphate
		Tri(dichlorisopropyl)phosphate
		
		PAHs (fuel combustion)
		Naphthalene
		Phenanthrene
		Anthracene
		Fluoranthene
		Pyrene
		Benzo(*a*)pyrene
		Benzo(e)pyrene
		Benz(*a*)anthracene
			Antioxidants
			2,6-di-tert-Butylphenol
			5-Methyl-1H-benzotriazole
			Butylatedhydroxyanisole (BHA)
			Butylatedhydroxytoluene (BHT)
			2,6-di-tert-Butyl-p-benzoquinone
			Others
			Tetrachloroethylene (*solvent*)
			Phenol (*disinfectant*)
			1,4-Dichlorobenzene (*fumigant*)
			Acetophenone (*fragrance*)
			p-Cresol (*wood preservative*)
			Phthalic anhydride (*used in plastics*)
			Bisphenol A (*used in polymers*)
			Triclosan (*antimicrobial disinfectant*)
			Petroleum Products:
			   n-alkanes
			   Hopanes and Steranes
			   UCM

Sex & Steroidal Hormones	**Biogenics**
	17*b*-Estradiol
	17*a*-Estradiol
	Estrone
	Estriol
	Testosterone
	Progesterone
		cis-Androsterone
		
		Pharmaceuticals
		Ovulation inhibitors:
		   17*a*-Ethynylestradiol
		   Mestranol
		   19-Norethisterone
			Hormone replacement therapy:
			   Equilenin
			   Equilin
			
			Sterols
			Cholesterol (*fecal indicator*)
			Coprostanol (*fecal indicator*)
			Stigmastanol (*plant sterol*)

Typical characteristic examples of gas chromatography-mass spectrometry (GC-MS) fingerprinting of some of these compounds are shown in Figure 2. Complete information about the contaminant chemical name, Composition and molecular weight are shown in Tables 2 and 3. Chemical structures of both aliphatic and aromatic hydrocarbons are shown in Figures 3 and 4, respectively.
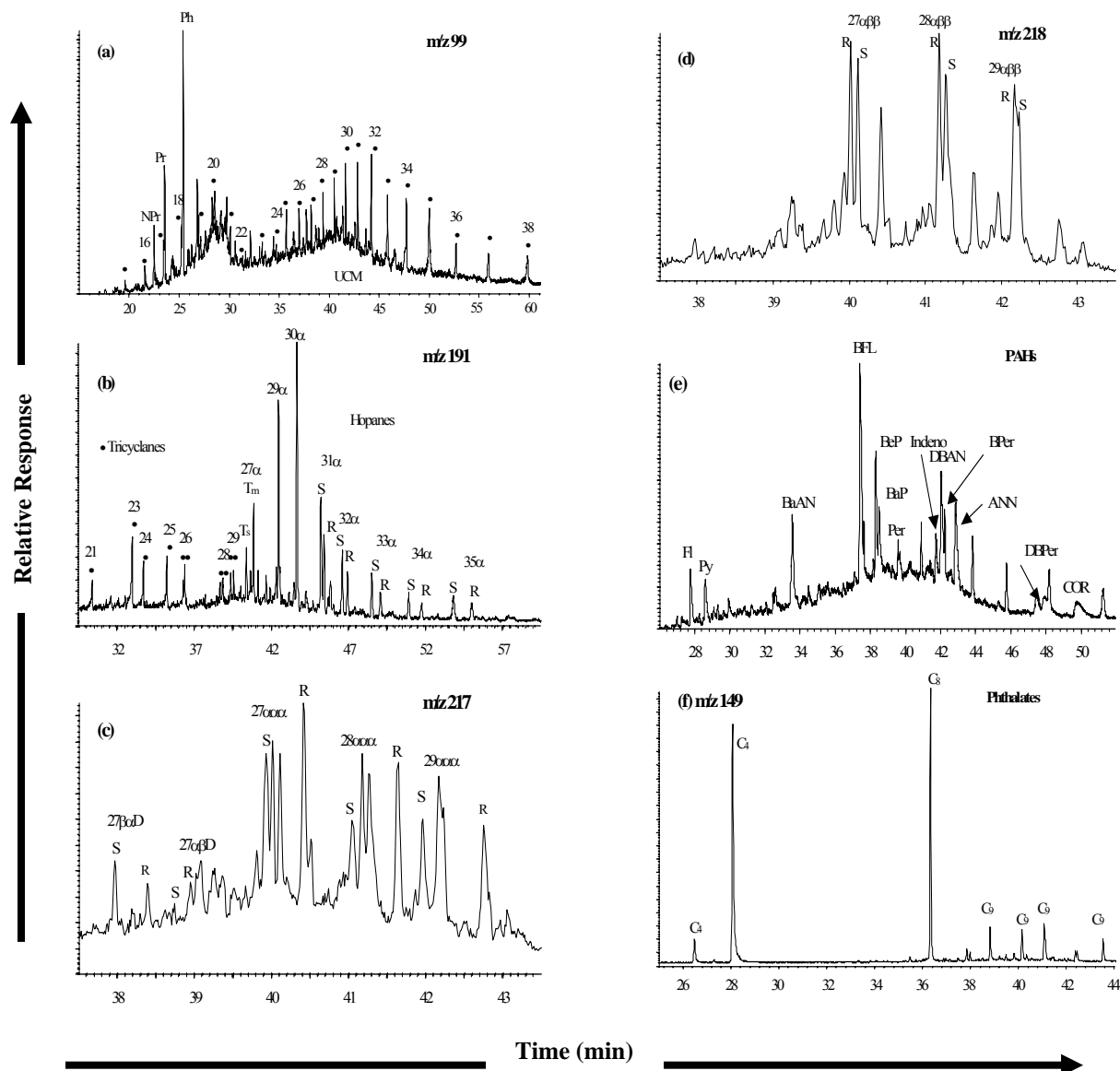

Figure 2. Typical GC-MS fingerprints for: (a) *n*-alkanes, m/z 99 (Pr = pristane, Ph = phytane, Npr = norpristane, UCM = unresolved complex mixture, numbers over peaks indicate carbon numbers); (b) hopane series, m/z 191; (c) ααα sterane series, m/z 217; (d) αββ sterane series, m/z 218; (e) PAH composition (Fl = fluoranthene, Py = pyrene, BaAN = benz[a]anthracene, BFL = benzo[b,k]fluoranthene, BeP = benzo[e]pyrene, BaP = benzo[a]pyrene, Per = perylene, Indeno = indeno[1,2,3-cd]pyrene, DBAN = dibenz[a,h]anthracene, BPer = benzo[ghi]perylene, ANN = anthanthrene, DBPer = dibenzo[ghi]perylene, Cor = coronene); and (f) C$_i$-phthalate esters, m/z 149 (i = carbon number of ester groups).
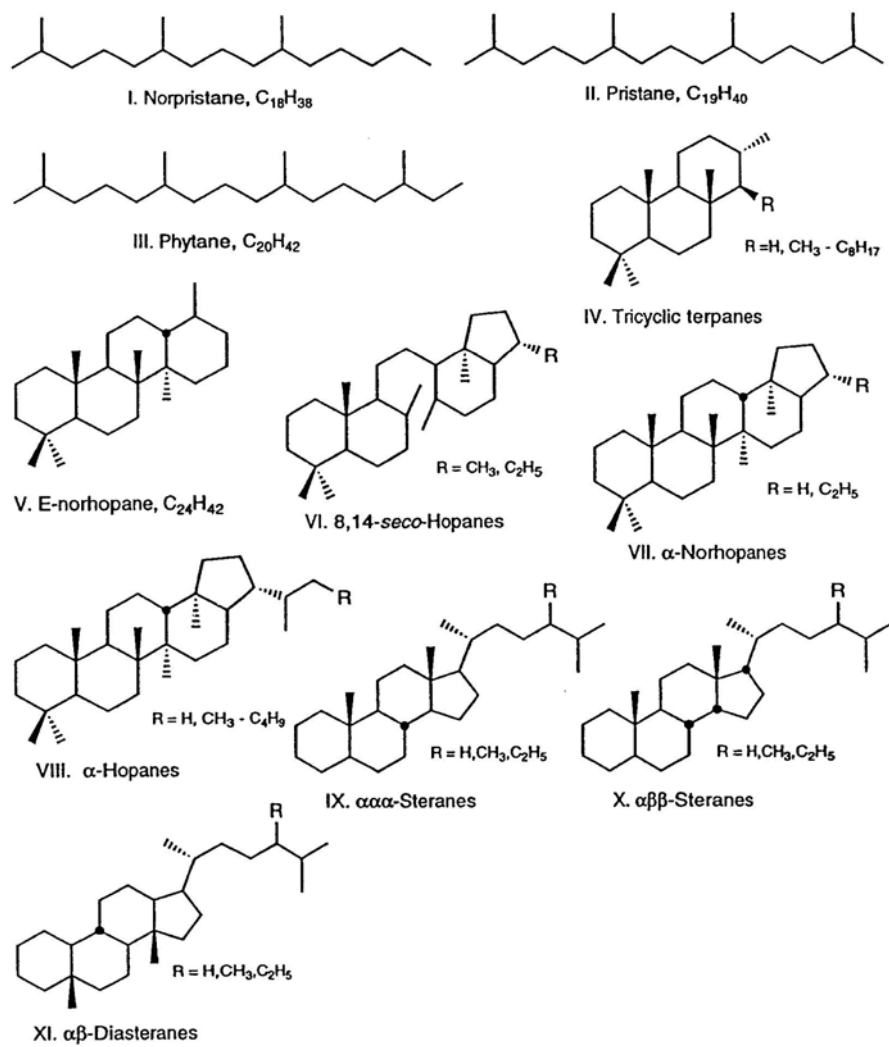
I. Norpristane, $C_{18}H_{38}$

II. Pristane, $C_{19}H_{40}$

III. Phytane, $C_{20}H_{42}$

IV. Tricyclic terpanes
R =H, CH_3 - C_8H_{17}

V. E-norhopane, $C_{24}H_{42}$

VI. 8,14-*seco*-Hopanes
R = CH_3, C_2H_5

VII. α-Norhopanes
R = H, C_2H_5

VIII. α-Hopanes
R = H, CH_3 - C_4H_9

IX. ααα-Steranes
R = H,CH_3,C_2H_5

X. αββ-Steranes
R = H,CH_3,C_2H_5

XI. αβ-Diasteranes
R = H,CH_3,C_2H_5

Figure 3. Chemical Structures of Some Aliphatic Hydrocarbons

I. Naphthalene

II. Phenanthrene

III. Anthracene

IV. Fluoranthene

V. Pyrene

VI. Benzo[b]fluorene

VII. Benz[a]anthracene

VIII. Chrysene

IX. Benzo[b]fluoranthene

X. Benzo[k]fluoranthene

XI. Benzo[e]pyrene

XII. Benzo[a]pyrene

XIII. Perylene

XIV. Indeno[1,2,3-cd]pyrene

XV. Benzo[ghi]perylene

XVI. Anthanthrene

XVII. Dibenz[a,h]anthracene

XVIII. Coronene

XIX. Dibenzo[a,e]pyrene
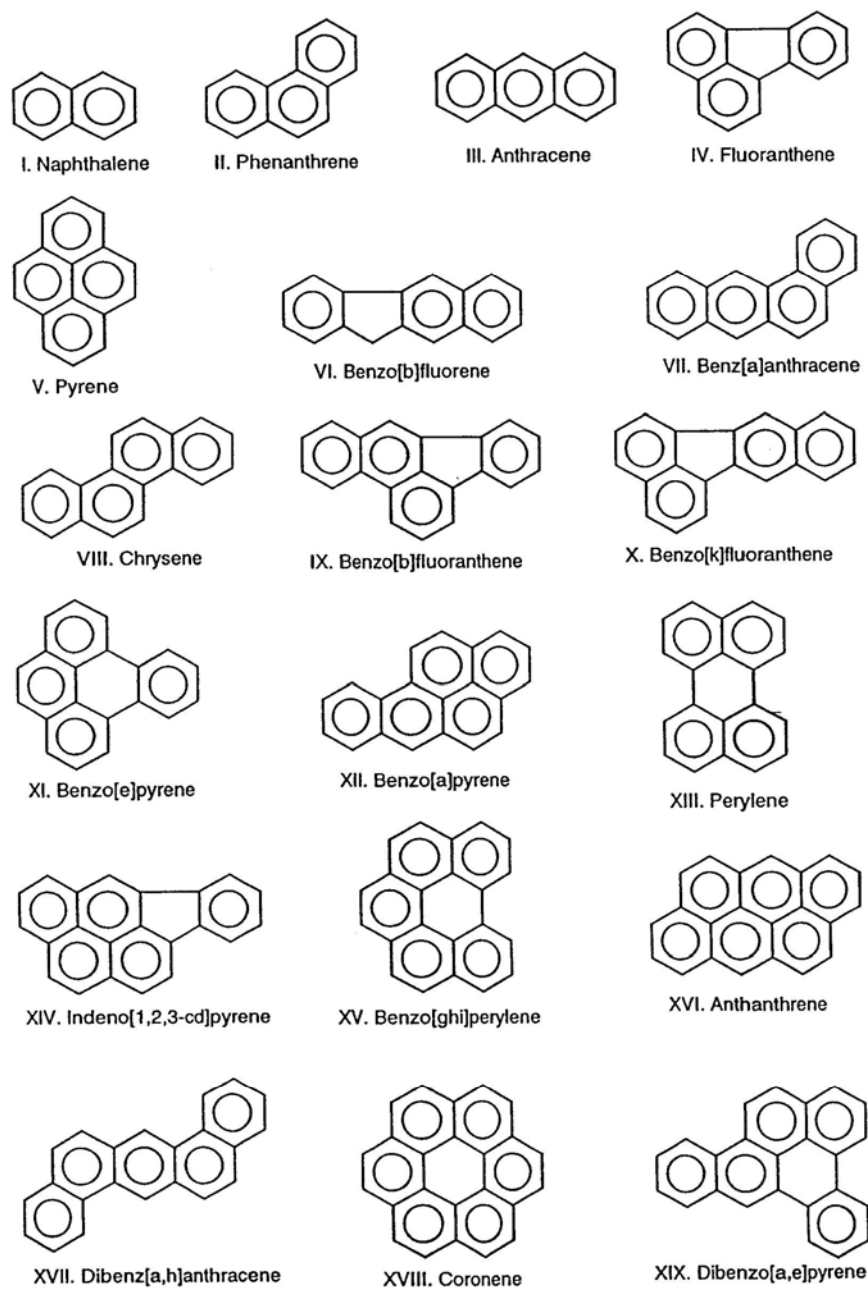
Figure 4. Chemical Structures of Some Polycyclic Aromatic Hydrocarbons

Based on the principal findings of the current investigation:

- Comprehensive research project was submitted to the United States Geologic Survey in March 2004. The project is entitled: "OCCURRENCE AND JOINT MULTICOMPONENT TOXIC/GENOTOXIC INTERACTION MODELING OF PHARMACEUTICALS, HORMONES AND OTHER ENDOCRINE DISRUPTING CHEMICALS IN THE WILLAMETTE RIVER", with Kenneth J. Williamson, and Bernd R.T. Simoneit (Co-Investigators), $399,537 for two years

- Collaboration or an interaction with the Port of Portland and City of Portland research teams has been established.

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